Inhibition of Substrate Oxidation by Endotoxin *in Vitro*[1] (34490)

MARTIN E. PLAUT AND JACK K. GOLDMAN
(Introduced by C. Bishop)
Department of Medicine, State University of New York at Buffalo and Veterans Administration Hospital, Buffalo, New York 14203

Bacterial endotoxins are lipopolysaccharides producing profound vascular changes in experimental animals (1). However, a number of studies indicate that endotoxin produces alterations in tissue metabolism independent of alterations in vascular resistance, blood flow, or tissue oxygenation (1–3). The present studies were undertaken to determine whether endotoxin itself might inhibit oxidative metabolism in liver, similar to the effects of staphylococcal alpha-toxin on toad bladder (4) and diphtheria toxin on myocardium (5).

Materials and Methods. E. coli, S. abortus equi, and *S. enteriditis* endotoxins (Difco Laboratories) were suspended in isotonic saline immediately before use. Radioactive substrates were obtained commercially in 98% or greater purity. All were dissolved in water and diluted with nonradioactive substrate. The dissolution of 1-^{14}C-palmitic acid was achieved by dropwise addition of NH_4OH to pH 9.0.

Mice weighing 20–25 g (Charles River Breeding Laboratories) were fed Purina Chow *ad libitum,* fasted 18 hr, and killed by a blow on the head. Livers were removed at once, blotted, weighed, and homogenized in 2.5 vol of oxygenated Krebs-Ringer phosphate buffer (pH 7.4). Aliquots of 0.25 ml of homogenate were added to 25-ml incubation flasks containing radioactive substrate, 2.0 ml oxygenated KRP, and 0.25 ml of either saline (control) or endotoxin in saline (experimental). Substrate concentrations were: 1-^{14}C-palmitate 100 μM, 1-^{14}C-hexanoate 100 μM, 2-^{14}C-pyruvate 4 mM, and 1,5-^{14}C-citrate 10 mM.

Flasks were sealed with rubber stoppers holding hanging plastic cups and incubated for 1 hr, with shaking, at 37°. At that time 0.25 ml of saline was injected into experimental flasks and 0.25 ml endotoxin (in saline) into control flasks. Hyamine (0.2 ml) was immediately injected into the plastic cups to collect the $^{14}CO_2$, and the medium was acidified by the injection of 0.3 ml of 4 N H_2SO_4. Incubation was resumed for 1 hr. The flasks were then opened, and the plastic cups were removed, wiped, and placed in counting vials. Counting was performed on a Nuclear Chicago Unilux II counter at an efficiency of 85% in 10 ml of toluene containing 3 g PPO and 0.6 g POPOP per liter.

Results. The oxidation of 1-^{14}C-palmitate to $^{14}CO_2$ was found to be progressively inhibited in a linear fashion as the concentration of *E. coli* endotoxin increased logarithmically. To determine whether this inhibition was due to carnitine depletion, the carnitine-independent oxidation of 1-^{14}C-hexanoate (6) was studied. Similar effects were noted, indicating that carnitine is not involved in the observed inhibition. Preferential inhibition of fatty acid oxidation was ruled out by demonstrating inhibition of $^{14}CO_2$ production using 2-^{14}C-pyruvate as substrate. Inhibition was again linearly related to endotoxin dose.

The oxidation of 1,5-^{14}C-citrate was next studied to determine whether entry of acetyl CoA into the Krebs cycle was blocked. Similar inhibition was observed indicating that the block is beyond the entry of substrate into the Krebs cycle. Results are similar for all four substrates and are illustrated in Fig. 1.

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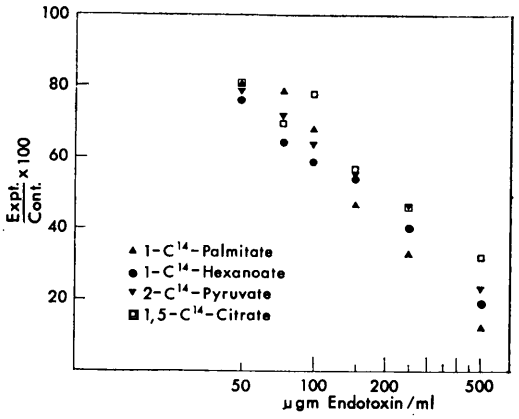

FIG. 1. Relationship of *E. coli* endotoxin concentration to inhibition of $^{14}CO_2$ production from several substrates.

Endotoxins from other forms of gram-negative bacteria, *S. enteriditis* and *S. abortus equi,* were subsequently studied. Production of $^{14}CO_2$ from 2-^{14}C-pyruvate was similarly inhibited regardless of the endotoxin used (Fig. 2). A highly purified *S. enteriditis* endotoxin (a generous gift of Dr. J. Rudbach of the Rocky Mountain Laboratory) has been tested and found to be active in this system.

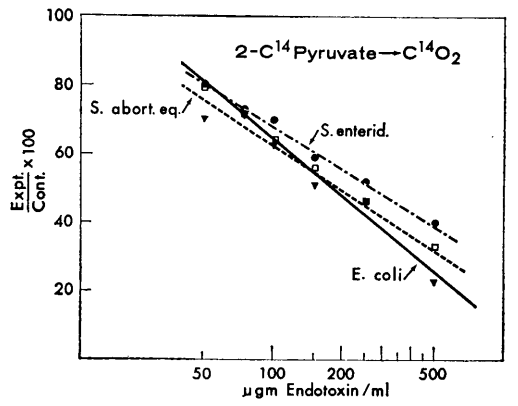

FIG. 2. Comparative inhibition of $^{14}CO_2$ production from 2-^{14}C-pyruvate by endotoxins from *E. coli* (▲), *S. enteriditis* (●), and *S. abortus equi* (□).

Discussion. These experiments demonstrate a dose-related inhibition of oxidative metabolism by a variety of bacterial endotoxins added *in vitro* to mouse liver homogenate. Under these conditions, the observed effects are independent of changes in circulatory dynamics and catecholamine or glucocorticoid levels. The site of action is beyond entry of metabolites into the Krebs cycle since the oxidation of citrate is inhibited as well as that of long- and short-chain fatty acids and pyruvate. This suggests inhibition of the Krebs cycle itself or the electron transport chain and its component cytochromes.

A recent study describes inhibition of mitochondrial electron transport and high-energy bond formation by endotoxin from *B. bronchiseptica* (7). The authors reported that *E. coli* endotoxin did not produce similar effects. Our results indicate that *E. coli* endotoxin does inhibit oxidative metabolism, and studies are underway to define the mechanism of this effect. Impairment of oxidative metabolism or phosphorylation may represent an effect common to many bacterial toxins.

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1. Rashova, H. and Vanecek, J., Pharmacol. Rev. **16**, 1 (1964).

2. Czeizel, E., Sajgo, M., Tarjan, Gy., and Kertia, P., Am. J. Obstet. Gynecol. **98**, 1129 (1967).

3. Graham, R. C., Jr., Karnovsky, M. L., and Shafer, A. W., J. Cell. Biol. **32**, 629 (1967).

4. Rahal, J. J., Jr., Plaut, M. E., and Weinstein, L., J. Clin. Invest. **47**, 1603 (1968).

5. Wittels, B. and Bressler, R., J. Clin. Invest. **43**, 630 (1964).

6. Fritz, I. B. and Yue, K. T. N., J. Lipid Res. **4**, 279 (1964).

7. Harris, R. A., Harris, D. L., and Green, D. E., Arch. Biochem. Biophys. **128**, 219 (1968).

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